# Rabitec

# Overview of efficacy studies

## 1. Introduction

Rabies, caused by the species Rabies virus (RABV) belonging to the genus Lyssavirus within the *Rhabdoviridae* family, is an acute, progressive viral encephalomyelitis that principally affects carnivores (worldwide) and bats (Americas), although any mammal can be affected. Globally, the dog is the most important reservoir, particularly in developing countries. In developed countries dog-mediated rabies has been largely eliminated by mass vaccination of dogs and other dog management measures.

However, rabies also has an important reservoir in certain wildlife species and effective control of rabies in these species is crucial to prevent re-introduction of rabies in the domestic dog population. In Europe, fox rabies predominated before its elimination by oral vaccination but in parts of Eastern Europe, rabies in raccoon dogs is of increasing concern.

Transmission almost always occurs via introduction of virus-laden saliva into tissues, usually by the bite of a rabid animal. This property is used for the oral vaccination of wildlife via baits that contain an immunogenic dose of a live attenuated strain of RABV.

### The vaccine

Rabitec is a live, attenuated aqueous suspension for oral vaccination of foxes and raccoon dogs.

Rabitec is indicated for the oral vaccination of raccoon dogs and foxes via baits.

### Indication

Active immunization of foxes and raccoon dogs against rabies to prevent infection and mortality among wildlife done under the direction of the responsible government agency.

Immunity is expected from 15 days after vaccination and lasts for at least 12 months.

### Recommended vaccination program and route of administration

The product is to be used under the direction of the competent authority.
The vaccine is administered via baits that are consumed by wildlife to be vaccinated.
The vaccine baits should be distributed preferably twice yearly, in spring and autumn, an annual distribution is also possible.
Depending on the epidemiological situation and the fox/raccoon dog density, the baits should be distributed with 18 to 30 vaccine baits / km². The higher distribution density can be necessary in areas with a large population of animals to be vaccinated.

In this document the data to support the efficacy of the vaccine for the indicated species are presented.

### Relevant legislation

The following monographs and guidelines have been taken into account:

- Ph. Eur. monograph 5.2.7: Evaluation of Efficacy of Veterinary Vaccines and Immunosera.
- Ph. Eur. monograph 0746: Rabies vaccine (live, oral) for foxes and raccoon dogs.

## 2. Laboratory trials

To investigate the efficacy of Rabitec the applicant performed the following studies per target species:

**Fox:**

- A challenge dose-finding study with graded doses ($10^{4.7}$, $10^{3.7}$, $10^{2.7}$, $10^{2.3}$ and $10^{2.0}$ $MICLD_{50}$ per ml).
- An orientating vaccination-challenge study in foxes vaccinated with slightly different vaccine doses ($10^{6.5}$ and $10^{6.7}$ FFU/ml) in blister volumes from 1.5 to 1.8 ml) and challenge with a very high dose. A detailed summary of the study is given in **Table 1.**
- Two pivotal immunogenicity trials in conformity with Ph. Eur. monograph 0746. The first study documenting a duration of immunity (DOI) of 6 months and an onset of immunity (OOI) of 15 days.
  The second study documenting a DOI of 12 months.
  Detailed summaries of these studies are given in **Tables 2 and 3.**

**Raccoon dog:**

- A challenge dose-finding study with graded doses ($10^{3.0}$, $10^{2.7}$ and $10^{2.3}$ $MICLD_{50}$ per ml).
- A pivotal immunogenicity trial in conformity with Ph. Eur monograph 0746. This study was valid as at the challenge dose of $10^{3.0}$ $MICLD_{50}$ per ml, 12 out of 12 (100%) control raccoon dogs were affected by the challenge. A detailed summary of the study is given in **Table 4.**

**Laboratory efficacy studies**

**Table 1. Orientating efficacy trial in foxes**

| Report / trial | Number / class of animals | Age at vaccination and route | Vaccine batch / control | Challenge | Efficacy parameters studied |
|---|---|---|---|---|---|
| VF-D/ Fu/0149/2014<br>Controlled laboratory vaccination-challenge trial | 3 groups:<br>• 6 vaccinates by bait (2 virus concentrations in 2 blister volumes)<br>• 4 vaccinates by direct oral application<br>• 2 Controls<br>Vaccinated groups in total 10 animals.<br>Control group 2 animals.<br>All animals sero-negative | Adult foxes by presenting a vaccine bait containing a blister filled with 1.5 or 1.8 ml vaccine.<br>The 4 foxes that did not take the bait received 1 ml vaccine by direct oral application. | - Rabitec<br>- batch TW_V00614<br>- $10^{6.7}$ FFU/ml -1.5 ml bait or<br>- $10^{6.5}$ FFU/ml -1.8 ml bait or<br>- $10^{6.5}$ FFU/ml -1.0 ml oral<br>- controls not treated | Intramuscularly, 54-56 days post vaccination with 2 x 0.4 ml containing $10^{4.7}$ $MICLD_{50}$ / ml of the virulent Rabies strain FLI ID 148. | • General health and mortality for 41 days post challenge<br>• Serological response after vaccination |

**Results**

- Both control foxes had to be euthanized at day 9 / 10 post challenge whereas all 6 foxes vaccinated via bait survived the observation period without clinical signs. Two out of the 3[1] foxes vaccinated by direct oral application also were protected. The third fox of this group was not protected and was euthanized at day 10.
- All 8 foxes that were protected had seroconverted (FAVN ≥ 0.5 IU/ml) at point of challenge. The fox that was vaccinated by direct oral application and was not found protected did not show seroconversion prior to the challenge.

**Conclusions**

- The study shows that a dose of at least $10^{6.8}$ per blister provides good protection against a high dose of pathogenic Rabies virus.
- The observed protection correlates 100% with seroconversion as measured by the FAVN test.

[1] On the day of the oral application of the vaccine it appeared that one animal had an ulcer in the jaw. On study day 24 the animal was unable to eat and was euthanized.

**Table 2. Immunogenicity trial in foxes, challenge 6 months post vaccination**

| Report / trial | Number / class of animals | Age at vaccination and route | Vaccine batch / control | Dose | Challenge | Efficacy parameters studied |
|---|---|---|---|---|---|---|
| VF-D/ Fu/0610/2014<br>Controlled laboratory vaccination-challenge trial | 2 groups<br><br>- Vaccinates<br>- Controls<br><br>Vaccinated group 30 animals.<br>Control group 12 animals.<br>All animals sero-negative. | At an age of 6-7 months by presenting a vaccine bait containing a blister filled with 1.7 ml vaccine | - Rabitec<br>- batch VM0010814-A<br>- $10^{6.6}$ FFU/ml<br><br>- control not treated | 1 vaccine bait per animal. | Intramuscularly, 190 days post vaccination with 2 x 0.5 ml containing $10^{0.7}$ $MICLD_{50}$ / ml of the virulent Rabies strain FLI ID 148. | • General health and mortality for 91 days post challenge<br>• At end of observation period absence of viral antigen in brain by FAT test<br>• Serological response after vaccination and challenge |

**Results**

- 12 out of 12 (100%) of the challenge control foxes died or were euthanized within 31 days post challenge whereas 28 out of 29[2] (97%) vaccinated foxes survived the observation period without clinical signs. One vaccinated animal was euthanized on day 34 due to clinical signs of Rabies.
- In all 12 control animals and in the non-protected vaccinated animal rabies viral antigen detected in brain samples. In none of the surviving animals viral antigen detected in the brain.
- 27 out of 30 (90%) vaccinated animals seroconverted (RFFIT ≥ 0.5 IU/ml) within 15 days post vaccination and another animal at 29 days. Two animals did not show seroconversion. One of these was the vaccinated animal that was not protected. All controls remained seronegative throughout the trial.

**Conclusions**

- The study complies with the pivotal immunogenicity test of Ph. Eur monograph 0746 and represents a valid trial.
- Based on seroconversion (RFFIT ≥ 0.5 IU/ml) an onset of immunity of 15 days has been demonstrated.
- Based on challenge a duration of immunity of 190 days (> 6 months) has been demonstrated.

[2] Prior to the challenge, one animal removed from the study for animal welfare reasons. No relation with the vaccination, no vaccine virus detected in medulla.

**Table 3. Immunogenicity trial in foxes, challenge 12 months post vaccination**

| Report / trial | Number / class of animals | Age at vaccination and route | Vaccine batch / control | Dose | Challenge | Efficacy parameters studied |
|---|---|---|---|---|---|---|
| A-R-D3/ Fu/1019/2017<br>Controlled laboratory vaccination-challenge trial | 2 groups<br>- Vaccinates<br>- Controls<br><br>Vaccinated group 26[3] successfully vaccinated animals.<br>Control group 14[4] animals.<br>All animals sero-negative. | At an age of 5-7 months by presenting a vaccine bait containing a blister filled with 1.7 ml vaccine | - Rabitec<br>- batch A-R-D3/Fu/ 1019/2017_Batch1<br>- $10^{6.6}$ FFU/ml<br><br>- control not treated | 1 vaccine bait per animal. | Intramuscularly, 53 weeks post vaccination with 2 x 0.5 ml containing $10^{3.0}$ $MICLD_{50}$ / ml of the virulent Rabies strain FLI ID 148. | • General health and mortality for 90 days post challenge<br>• At end of observation period absence of viral antigen in brain by FAT and or RT-PCR test<br>• Serological response after vaccination and challenge |

**Results**

- 13 out of 14 (93%) of the challenge control foxes died or were euthanized within 17 days post challenge whereas 26 out of 26 (100%) successfully vaccinated foxes survived the observation period without clinical signs.
- In 13 of 14 control animals, rabies viral antigen and/or rabies viral RNA was detected in brain samples. In none of the 26 successfully vaccinated animals viral antigen or RNA detected in the brain.
- 25 out of 26 (96%) successfully vaccinated animals seroconverted (RFFIT ≥ 0.5 IU/ml) post vaccination, the other animal responded with a RFFIT of 0.4 IU/ml.
- All 26 successfully vaccinated animals seroconverted in the ELISA post vaccination.
- The 3 animals that were not successfully vaccinated did not seroconvert in the RFFIT (< 0.3 IU/ml) nor in the ELISA (-).
- Four control animals were tested positive at 1 or 2 occasions in the RFFIT (0.39 – 0.74 IU/ml). This is considered a-specific due to serum toxicity as all controls remained seronegative until challenge in the ELISA at all test points throughout the trial.

**Conclusions**

- The study complies with the pivotal immunogenicity test of Ph. Eur monograph 0746 and represents a valid trial.
- Based on challenge a duration of immunity of at least 1 year has been demonstrated.

[3] The group started with 31 animals but 2 animals dropped out prior to the challenge for reasons not related to the vaccination and 3 animals were not successfully vaccinated (retrospectively they did not met the inclusion criteria).

[4] The group started with 15 animals but 1 animal dropped out prior to challenge.

**Table 4. Immunogenicity trial in raccoon dogs**

| Report / trial | Number / class of animals | Age at vaccination and route | Vaccine batch / control | Dose | Challenge | Efficacy parameters studied |
|---|---|---|---|---|---|---|
| VF-D/Mh/ 0692/2015<br>Controlled laboratory vaccination-challenge trial | 2 groups<br>- Vaccinates<br>- Controls<br><br>Vaccinated group 28 animals vaccinated by bait) (+8 by DoA).<br>Control group 12 animals.<br>All animals sero-negative. | At an age of 7-8 months by presenting a vaccine bait containing a blister filled with 1.7 ml vaccine (28 animals)<br>(+ an additional 8 animals per DoA.with 1.7 ml vaccine) | - Rabitec<br>- batch VM0020814-A<br>- $10^{6.6}$ FFU/ml<br><br>- control not treated | 1 vaccine bait per animal<br><br>(or 1.7 ml per DoA). | Intramuscularly, 183/184 days post vaccination with 2 x 0.5 ml containing $10^{3.0}$ $MICLD_{50}$ / ml of the virulent Rabies strain FLI ID 148. | • General health and mortality for 92 days post challenge<br>• At end of observation period absence of viral antigen in brain by FAT test<br>• Serological response after vaccination and challenge |

**Results**

- 12 out of 12 (100%) of the challenge control raccoon dogs euthanized/died during the first 15 days post challenge.
- In all 12 control animals rabies viral antigen detected in brain samples.
- 25 out of 28 (89%) raccoon dogs vaccinated by bait seroconverted (RFFIT ≥ 0.5 IU/ml) within 13/15 days post vaccination. The other 3 animals seroconverted throughout the study.
- 28 out of 28 (100%) raccoon dogs vaccinated by bait survived the observation period without clinical signs. In none of these 28 animals viral antigen was detected in brain.

- 8 out of 8 (100%) raccoon dogs vaccinated by direct oral application (DoA) seroconverted (RFFIT ≥ 0.5 IU/ml) within 13/15 days post vaccination.
- 8 out of 8 (100%) raccoon dogs vaccinated by DoA survived the observation period without clinical signs. In none of these 8 animals viral antigen was detected in brain.

**Conclusions**

- The study complies with the pivotal Immunogenicity test of Ph. Eur monograph 0746 and represents a valid trial.
- Based on seroconversion (RFFIT ≥ 0.5 IU/ml) an onset of immunity of 15 days has been demonstrated.
- Based on challenge a duration of immunity of 183/184 days (> 6 months) has been demonstrated.

### Duration of immunity

The interval between vaccination and challenge in the pivotal efficacy trials in foxes and raccoon dogs was at least 180 days as guided by Ph. Eur monograph 0746. Further, this interval was extended to 53 weeks in the duration of immunity study in foxes. Based on bio-equivalence in the affect rates and protections rates at 6 months in foxes and raccoon dogs it was concluded that the data in foxes might be extrapolated to the even more minor species, raccoon dogs. This was further confirmed by an identical VNA-kinetics and ELISA-kinetics during the first 6 months post-vaccination with SPBN GASGAS.

It is therefore concluded that the efficacy 12 months post vaccination of SPBN GASGAS in foxes is also applicable for raccoon dogs. For animals welfare reasons it was not justified to perform an additional 15 months duration study in raccoon dogs.

### Discussion and conclusion

As shown in these studies the vaccine virus SPBN GASGAS (active ingredient of Rabitec) when given as recommended (via bait) at the minimum dose ($10^{6.6}$ FFU/ml in a volume of 1.7 ml) protected vaccinated animals against a lethal dose of the virulent challenge virus strain FLI ID 148.
An onset of immunity of 15 days has been demonstrated.
A duration of immunity of at least 53 weeks was demonstrated.
The serological data confirm the good immunogenicity of the vaccine.

## 3. Field trials

In accordance with the EMA Scientific Advice obtained before submission for Marketing Authorization in the EU, no field trials have been performed. After obtaining Marketing Authorization this vaccine will be used under governmental supervision.

List of abbreviations

| | |
|---|---|
| DoA | Direct oral Application |
| FAT | Fluorescent Antibody Test |
| FAVN | Fluorescent Antibody Virus Neutralization Test |
| FFU | Focus Forming Units |
| FLI | Friedrich Loeffler Institute |
| IU | International Units |
| $MICLD_{50}$ | Mice Intracerebral Lethal Dose 50% |
| RABV | Rabies virus |
| RFFIT | Rapid Fluorescent Foci Inhibition Test |
| VNA | Virus Neutralizing antibodies |